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Laboratory Studies *in* Biology

Observations and their Implications

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To the Student

If you have ever had a laboratory course in science, the work you will do in this course will probably come as a surprise to you. Typically, in a teaching laboratory, the emphasis is on verification; here it will be on discovery.

"Discovery of what?" you may ask. "How can I, a mere beginner, discover a new fact of Nature?" You are right; it is unlikely (though not impossible) that you will, in the short time available, discover a new truth in a field so long cultivated. Then what may you expect to discover?

Suppose you were asked: "What shape is the earth?" You would probably answer, "Round." But suppose the first question were followed by a second: "*How do you know?*" What would you say then? The second question is not an easy one. In trying to answer it you would discover a great deal—about the elementary observations ("facts," in one sense) on which is based your belief in the "fact" (in another sense) that the earth is round; about your methods of reasoning; about your motives in choosing from among alternative hypotheses:—in a word, you would discover a great deal about yourself. Such is the sort of discoveries you should hope to make in this course.

You already know quite a bit about biology. You know something of how the human body works, how plants differ from animals, and perhaps even something of genes and enzymes. Knowledge of these matters is in the air these days. Frequently you might be able to answer the questions in your manual without carrying out the observations and experiments called for —in other words, you could "dry-lab" the work. *But you had better not.* There is a structure to scientific knowledge that you can appreciate well only if you carry out the steps needed to develop it, with some deliberation, with the material before you. Furthermore, you will frequently find that the "obvious" answer to a question is not the correct answer at all. Such questions are not deliberately put in to serve as traps; rather they are here to bring home to you the truth that *science is a self-correcting system for obtaining knowledge.* The history of science is the history of making errors—and correcting them. Science is an activity. From each new hypothesis many deductions are made. These are tested by observation and experiment, and when found to be false (as they almost always are, at least in part) new hypotheses are developed, to be tested again. And so on, ad infinitum. The concept of infallibility has no place in the scientific laboratory. Scientists expect to make errors; they hope also to correct them. That this approach to the realities of the world has proven fruitful is now painfully obvious. As Emile Duclaux, one of Pasteur's most perceptive students, said: "It is precisely because science is never sure of anything that it always advances."

Time after time you will be asked to make hypotheses. Always do so. Do not be afraid of making wrong guesses. Most hypotheses are wrong. You should keep in mind what George Sarton has said of learning a foreign language: "Nobody will ever speak a language well if he lacks the humility to speak it badly." The same can be said of learning science. Activity (and that includes intellectual activity) must come before criticism and evaluation.

In arriving at your answers, try to think and work independently—but not alone. Constantly test the results of your thinking by exposing them to the criticism of your fellow-students. When you and your colleagues cannot agree, consult your instructor: he is there to help you. The self-correcting feature of scientific method strongly depends on the fact that science is not a solo performance. *Science is a social activity.*

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1. The Microscope

A. Introduction

The progress of science depends on both tools and ideas. Laboratory instruction in biological science is designed to make you familiar with some of the more important tools and show you how these tools are used to explore ideas, thus answering old questions and generating new ones. For more than two centuries, the principal tool of the laboratory biologist has been the microscope. Through its use, biologists of the 19th century arrived at that great milestone in the analytical study of living things, the cell theory. We shall presently study some of the evidence that supports this theory, but before doing so we need to become familiar with the tool that we are going to use, the microscope.

Optical miscroscopes are of two kinds, *simple microscopes* and *compound microscopes.* A simple microscope has but a single lens, like the familiar magnifying glass. Such lenses are usually of low power, typically magnifying each linear dimension about ten times (10×); but it is possible to make tiny, highly curved lenses that magnify much more. The 17th century biologist Leeuwenhoek carried out his pioneering studies using simple microscopes with magnifying powers of some 200×.

Compound microscopes, by contrast, have two or more lenses in series to produce the image. Useful magnifications as great as 1800× are obtainable with such instruments. Most basic biological observations can be made, however, with much less magnification. The microscope you will use offers a choice of two most useful magnifications, one of about 100×, and one of about 400×.

NOTE. *In the laboratory exercises you are called upon to answer many questions. Thoughtfully prepared answers, written out in sufficient detail, will be of great aid to you in review later. Often you will find that a quick sketch done in the margin of the page will help even more. By all means, make such sketches, even though they are not explicitly demanded of you: by so doing, you will see better, learn more, and recall easier.*

B. Parts of the Microscope

Examine the microscope, comparing it with Figure 1-1. *Learn the names of the parts.*

A microscope is an expensive instrument that can be easily damaged by improper handling. However, if you meticulously follow the rules given by your manual and by your instructor, there is little chance of your harming the instrument. The most important "Do's and Don'ts" are illustrated in Figure 1-2.

At the start of each day's work, wipe the outside surfaces of both ocular and objective lenses with lens paper. **Do not remove any lenses unless given permission by the instructor.** Never touch a clean lens with your bare finger, for the oil from your skin will blur the image.

As directed by your instructor, adjust the light so that it enters the tube of the microscope. Now, while looking into the ocular, rotate the nosepiece so that first one objective then the other is in line with the body tube. [1] By feel, how can you tell when this alignment is correct? .....

.....

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* The initials refer to *Biology Its Human Implications* (Second Edition) by Garrett Hardin, W. H. Freeman and Company, San Francisco.

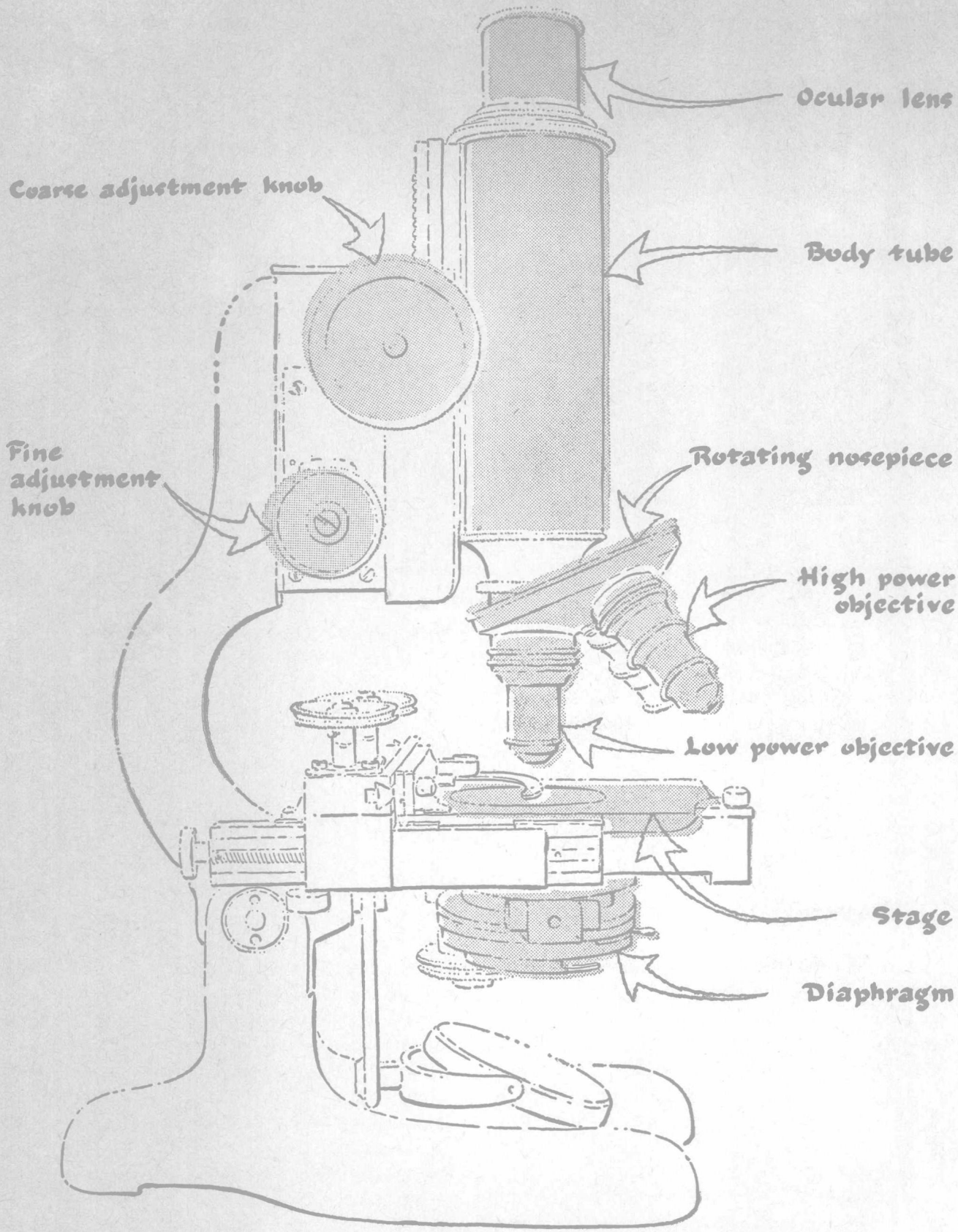


FIG. 1-1. *A Compound Microscope.*

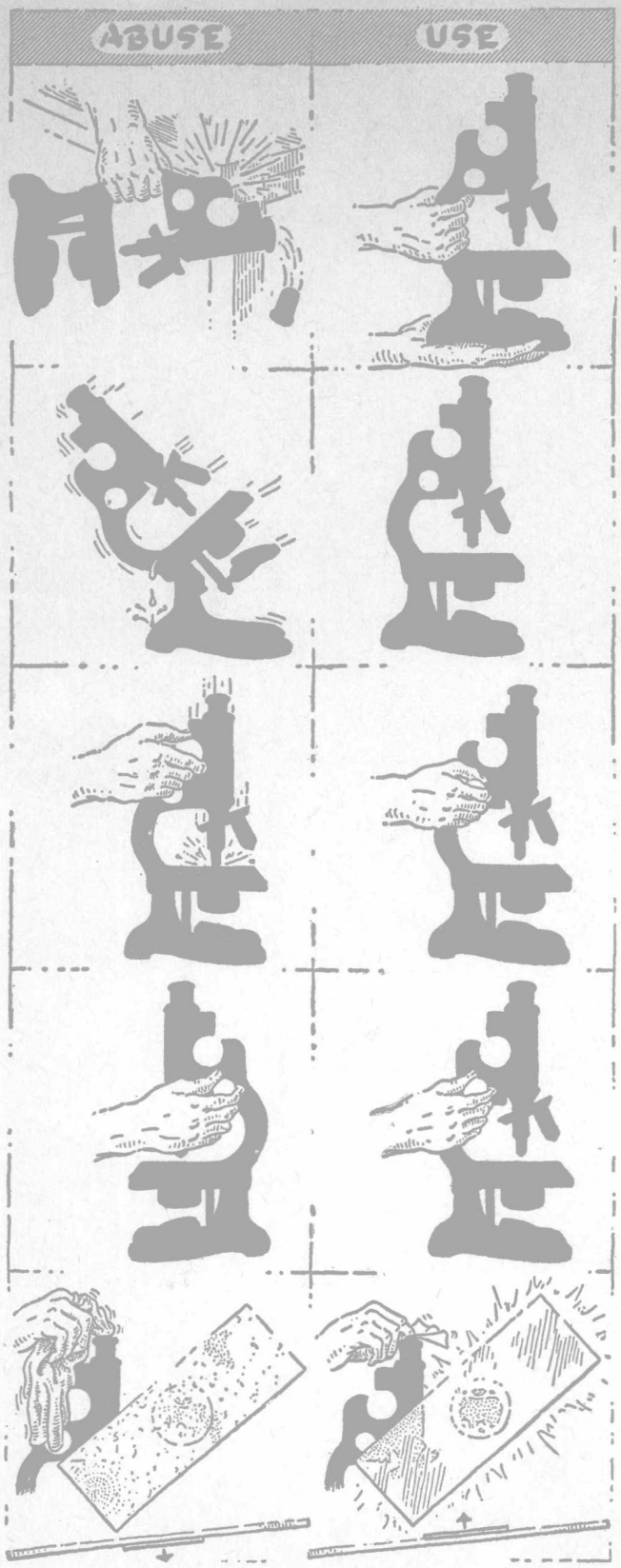


FIG. 1-2. *Abuse and Use of the Microscope.*

Notice that the visual field, as you look in the ocular, is brighter with one objective than it is with the other. The brighter visual field is produced by the lower-powered objective.

Now look at the figures engraved on the barrel of each objective. [2] What significant figures are found on the low power? .....

..... [3] On the high power?

.....

With the objective lens at least ⅛ inch from the microscope stage, rotate the coarse adjustment knob through one full turn. [4] About how far does this cause the body tube to move? .....

.....

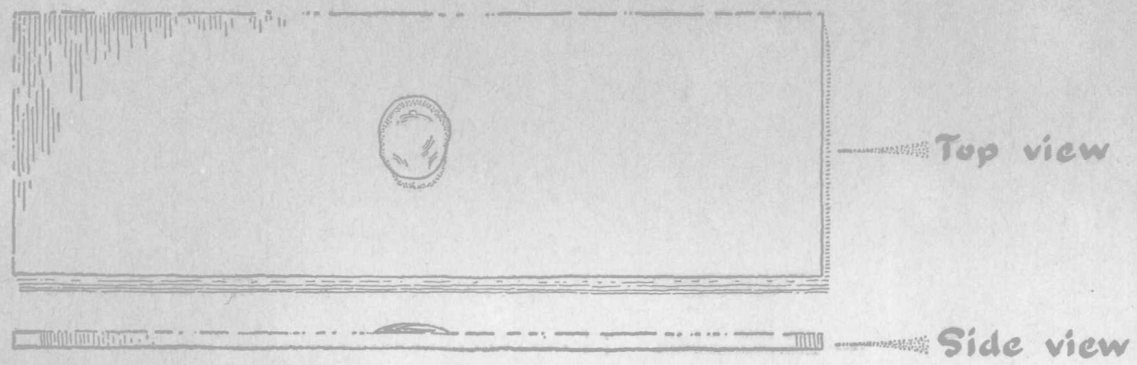
The fine adjustment mechanism, like an automobile steering wheel, has a limited turning range. It is most convenient to manipulate it in the middle of its range. [5] One complete turn of the fine adjustment knob moves the tube about how far? .....

C. Use of the Microscope

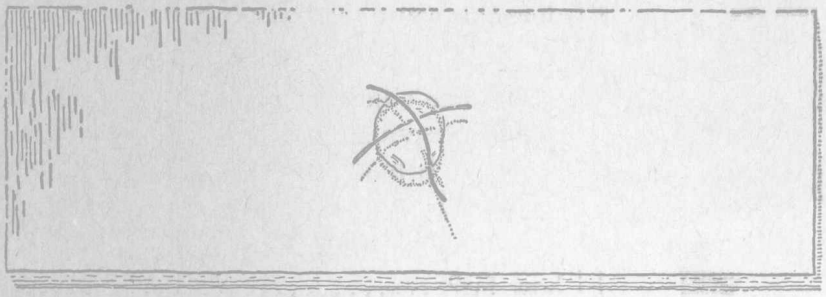
Material that is to be looked at with the microscope is best mounted on a microscope **slide,** a piece of thin glass 1 × 3 inches, and covered by a smaller, even thinner piece of glass called a **cover glass** or a **coverslip.** Both slide and cover glass should first be washed with water and then dried with cheesecloth, making sure that no lint is left on them. Set slide and cover glass on a clean, dust-free surface until needed.

Place a **small** drop of water in the center of the slide by touching the dropper from a water bottle to it. (See Figure 1-3.) From agreeable fellow-students secure two hairs, one blond and one brunette. Using forceps, place a half inch piece of each hair on the slide so that they cross in the center of the drop of water. Lower the cover glass so as to trap as few air bubbles as possible.

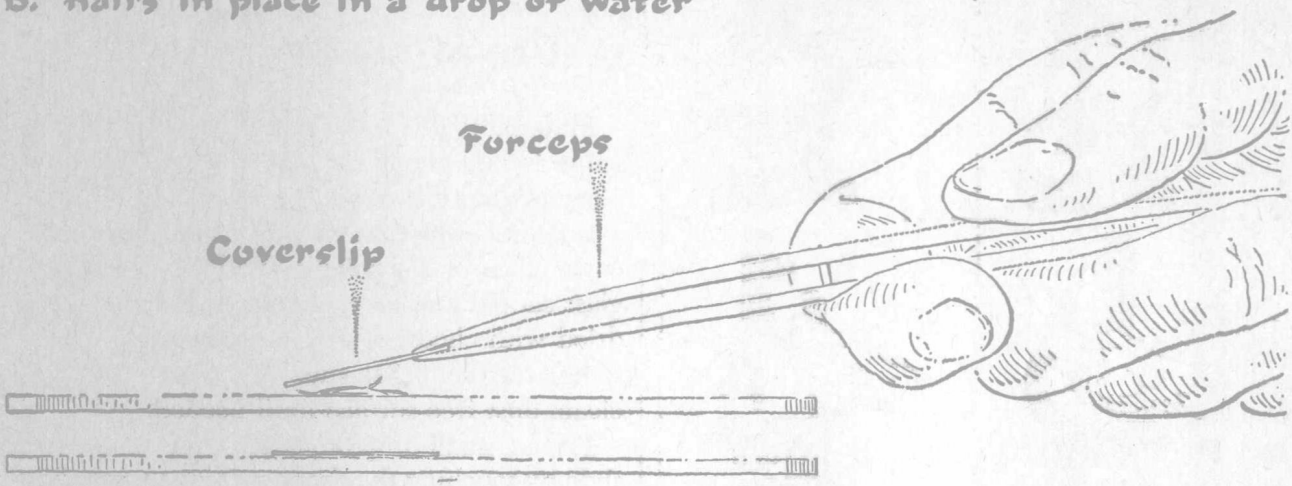
Place the slide on the stage of your microscope and adjust the light so that it is shining through the mounted material. Swing the low power objective into position for use, being careful not to allow it to touch the cover glass. **While watching from the side turn the coarse adjustment until the objective is about ¼ inch from the cover glass.**



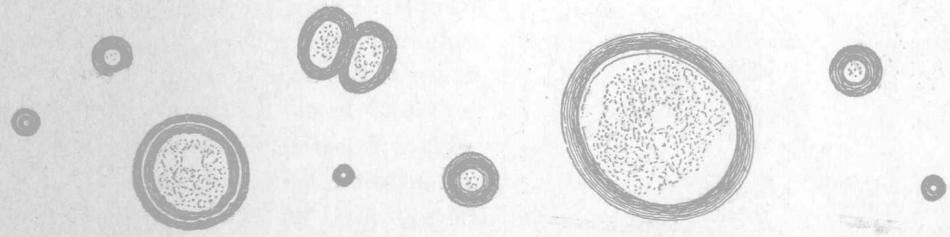
A. A slide with a drop of water in place



B. Hairs in place in a drop of water



C. Placing the coverslip — and coverslip in place



D. Air bubbles as they appear under the microscope

FIG. 1-3. *Preparation of a Water Mount on a Microscope Slide.*

Now look into the ocular and **slowly raise** the objective, using the coarse adjustment, until the hairs come into view. Center the crossed hairs in the field of view by moving the slide with your fingers. [6] As you push the slide to the right, in which direction do the hairs appear to move?

.....

Raise the objective with the fine adjustment until the hairs are just out of focus. Now lower the objective slowly. [7] Which hair comes into focus first? ..... [8] When the top hair is in best focus is the lower one at all visible? ..... [9] When the bottom hair is in focus, is the upper one at all visible? .....

A perfectly prepared slide has no entrapped air bubbles. But if your first slide is less than perfect, it is just as well, for you need to learn early what an air bubble looks like under the microscope. Otherwise you may later think you have discovered some wonderful new species of organism! Before looking at the hairs further, find some air bubbles and study them carefully, using the fine adjustment to focus on various levels. Note the characteristic shading of the edges of air bubbles. You should recognize them for what they are whenever you see them again.

You are now ready to examine the hairs under high power; but first, some words of caution. Every modern microscope is supposed to be *parfocal,* that is, so constructed that the object will be in focus with the high power, without any further adjustment of the body tube, if focus has first been secured with low power. However, until you have verified that your instrument is parfocal, you need to proceed with extra caution.

Still using low power, focus again on the crossed hairs and center them in the field. Now, as you watch from the side, swing the high power into position, seeing to it that it does not scrape the cover glass. This operation satisfactorily completed, look into the ocular. If the hairs are in focus, fine; if not, you will have to adjust the focus.

When using high power, focus only with the fine adjustment.

The high power objective clears the cover glass by so little that there is always danger of breaking the cover glass, and—much worse—damaging the lens. If your instrument is not parfocal, consult your instructor about the proper procedure before going on.

[10] When you have the top hair in focus with the high power lens, is the bottom one also in focus? ..... [11] Is it visible? ..... [12] What is the appearance of the surface of the blond hair? .....

[13] The dark hair? .....

[14] Are the edges of the blond hair in focus at the same time its surface is in focus? .....

[15] Is the depth of field greater or less than half the diameter of the blond hair? .....

[16] What enables you to decide this? .....

.....

.....

..... [17] Does the internal structure of the blond hair differ from that of the dark hair? ..... [18] Describe. .....

.....

.....

.....

Since under high power the surface of the hair is not in focus at the same time as the outer edges are in focus, you can understand that the depth of field is very shallow. When focused on the edges of the hair, the upper surface is out of focus and you see what is called an **optical section** of the hair.

It is understood from these observations that a sense of depth in the field of the microscope can be secured by using the fine adjustment. Your mind must take an active part in observation. As you focus while looking in the microscope you get a series of two-dimensional pictures which your mind integrates to give you a three-dimensional concept. This is so important that persons skilled in the use of the microscope automatically take hold of and use the fine adjustment every time they look into a microscope.

D. Use of the Microscope

Mount on a slide, by the same method used to mount the hairs, a piece of very small glass tubing and on another slide a very small piece of glass rod. The tubing is hollow and the rod is solid. One is in a dish labeled A and the other in a dish B. These are about .02 mm. (approximately 1/1000 inch) in diameter. Determine by observing under the microscope whether A or B is the glass rod or the tubing. Locate these objects with low power then observe with high power.

[19] Which one, A or B, is the tubing? .....

..... [20] What observations led you to this conclusion? .....

.....

[21] Was it necessary to use the fine adjustment screw in making your observations? .....

[22] What observations enabled you to identify the glass rod? .....

.....

..... [23] Was it necessary to use the fine adjustment screw to get this evidence?

..... [24] Is it possible to get an accurate, three dimensional concept of these small structures without using the fine adjustment screw? .....

E. Use of the Microscope

Observe a prepared slide of a fly's wing under the high power of the microscope. Take great care in using the high-power objective because wrong adjusting will cause the objective to strike and break the slide. Answer the following questions while you make your observations.

NOTE: *Always keep the cover glass uppermost when observing microscope slides.*

[25] Are there hairs on the wing? .....

[26] Do the veins in the wing have hairs on them?

.....

[27] What is the color of the hairs? .....

[28] What is the shape of the hairs? .....

[29] Are all of the hairs in a field in focus at the same time? ..... [30] Observe one hair on the membrane near the center of the wing. Is all of it in focus at one time? .....

[31] Is all of it visible at one time? .....

[32] Are there hairs on both the upper and lower surface of the wings? ..... [33] What is the difference in shape or color of those on the lower surface as compared with those on the upper? ..... [34] What are the means of determining which hairs are on the upper surface and which are on the lower surface?

..... [35] When focused on the tip of a hair only, can you determine whether the hair is growing from the upper or the lower surface of the wing? ..... Try this to make sure. [36] Are there hairs on the underside of the large veins? ..... [37] What property of the material under observation is necessary before the underside of a structure can be observed with the microscope without turning the material over? ..... [38] Are the hairs at the margin of the wing horizontal, vertical, or otherwise? .....

[39] What physical operation and what mental process must work together in order to get a three-dimensional concept when observing with the microscope? .....

.....

F. Magnification and Inversion

Observe with low power of the microscope the millimeter (mm.) divisions on a transparent or translucent ruler. [40] Approximately what is the diameter of the field of the low power objective? ..... mm. (1 mm. = 1000 microns). [41] What is the diameter of the low power field in microns? .....

Switch to high power and observe the mm. scale again. [42] Can you see two divisions in one field? ..... Carefully slide the scale so the right edge of the field is approximately in the middle of one of the lines on the scale. Note a blemish or bit of dirt at the opposite edge of the field. Slide the ruler very carefully so that this speck of dirt moves across the field to the opposite edge. Find a second bit of dirt at the opposite edge and repeat the operation by moving the ruler in the same direction as before. Continue this until the middle of the next division line on the scale reaches the edge of the field. From these observations you can estimate the diameter of the high power field. [43] Its diameter is what fraction of a mm.? ..... [44] What is the diameter of the field in microns? ..... [45] If the length of an object is 1/10 the diameter of the lower power field, what is the length of the object in microns? ..... [46] If the length of an object is 1/5 the diameter of the high power field, what is its length in microns? ..... [47] If 30 cells in a row extend across the high power field, what is the average width of one cell? .....

Under low power observe one of the numbers on the scale. [48] Is it in the same position as when viewed with the naked eye? ..... [49] Is it reversed, turned through 90 degrees, or inverted and reversed? .... This inversion of the image is due to the crossing of the rays of light as they come through the microscope.

2. The Cell

A. Introduction

In carrying out the laboratory work described in this exercise you should have two purposes in mind: first, to observe various kinds of cells, both living and dead; and second, to learn to appreciate what is meant by the word "observe."

But, you may ask, is not the word "observe" a synonym for "see"? Is not the former merely a fancy substitute for the latter? Not at all: not as scientists use the term. When you are asked to *observe* an object or process, you are asked to do something more than merely *see* it. What is this something more? Arriving at a full answer to this question will take time—perhaps the rest of your life, if you will use it so—but (fortunately) we can suggest a first approximation now, before you start to look at cells. Morris R. Cohen, a 20th century American philosopher, has put the problem thus:

> Leeuwenhoek was, undoubtedly, a keenly interested and close observer, because he was looking for definite things. The ordinary man can look through a microscope but he doesn't see anything of importance. I know, for I looked through microscopes when I was a student, and could not see anything except patches of what looked like mud. I was told to draw what I saw, but I couldn't draw anything definite when I saw nothing definite. Some ideas are required before you can have really intelligent observation. We see with our mind's eye as well as with our physical eye. . . .*

The word observe, as we shall use it, implies this "seeing with the mind's eye," as well as with the physical eye.

Using your microscope according to the procedures learned in Study 1, you will observe different kinds of plant and animal cells. To help you in this activity, numerous questions are asked of you. Look **and think** before you reply to them. Keep in mind the old Arab proverb: **The eye is blind to what the mind does not see.**

B. The Parts of Metabolic Cells

Elodea (also known as Anacharis) is a flowering plant which grows submerged in fresh water. (See Figure 2-1.) It is useful for laboratory work because its leaves are thin and simple in structure.

An Elodea leaf can be observed in the living condition by removing it from the plant, mounting it in a drop of water on a slide with a cover slip, and examining it under the microscope. Important: keep the mounted leaf surrounded by water, or it will die. When water begins to evaporate from under the cover slip, it can easily be replaced (See Figure 2-2) by placing the end of a dropper of water on the slide at the edge of the cover slip and squeezing out a little water. Capillarity will draw the water under the cover slip until the space is completely filled.

The leaves from a bud (Figure 2-1 B) are easiest to examine because they contain fewer **chloroplasts**, the bodies in green-plant cells that contain the green pigment.

Mount one of the bud leaves. Examine with the low power of the microscope. Examine the entire

* From Morris R. Cohen, *Studies in Philosophy and Science*. N. Y.: Henry Holt and Co. 1949. By kind permission of the copyright owners.